PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
860		
International application No. PCT/IL2004/000314	International filing date *(day/month/year)* 07.04.2004	Priority date *(day/month/year)* 08.04.2003
International Patent Classification (IPC) or national classification and IPC C12N5/06, A61K38/48, C12N9/64		
Applicant YEDA RESEARCH AND DEVELOPMENT CO. LTD et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 13 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08.11.2004	21.11.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Morawetz, R Telephone No. +49 89 2399-8155

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IL2004/000314

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-42 as originally filed

Claims, Numbers

1-62 as originally filed

Drawings, Sheets

1/5-5/5 as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-3,5-12,14-32,34-40, 54-56, 58-60 (all partially)

because:

☒ the said international application, or the said claims Nos. 10-17,30-40,54-58 relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 1-3,5-12,14-32,34-40, 54-56, 58-60 are so unclear that no meaningful opinion could be formed *(specify)*:

see separate sheet

☒ the claims, or said claims Nos. 1-3,5-12,14-32,34-40, 54-56, 58-60 are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 1-3,5-12,14-32,34-40, 54-56, 58-60

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☒ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	19-40, 47-53, 55-58
	No:	Claims	1-18, 41-46, 54, 59-62
Inventive step (IS)	Yes:	Claims	
	No:	Claims	19-40, 47-53, 55-58
Industrial applicability (IA)	Yes:	Claims	1-9,18-29,41-53, 59-62
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1.1. Present claims 1-9 relate to a method defined by reference to a desirable characteristic or property, namely a method of increasing sensitivity of stem cells to a chemoattractant, the method comprising exposing the stem cells to a matrix metalloprotease or an active portion thereof, which is capable of increasing a level of at least one chemoattractant receptor of the stem cells to thereby increase the sensitivity of the stem cells to the chemoattractant. The claims cover all methods having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such methods, namely the methods wherein the metalloprotease is selected from MMP-2 and MMP-9 and the chemoattractant receptor is CXCR4. The same objection applies mutatis mutandis to claims 10-40 and 54-62. The claims thus lack support, and the application lacks disclosure. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the method by reference to a result to be achieved. Consequently, no opinion will be established for claims 1-40 and 54-62 insofar as they do not relate to MMP-2 or MMP-9 and CXCR4.

1.2. Claims 10-17, 30-40, 54-58 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item IV

Lack of unity of invention

1. Rule 13 PCT stipulates that the international application shall relate to one invention only or to a group so linked as to form a single general inventive concept. Where a group of inventions is claimed in one and the same international application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding "special technical features", i.e. technical features that define a novel and inventive contribution over the prior art (Rule 13.2 PCT).

2. The common concept (technical relationship) linking the present claims together is that they all are concerned with matrix metalloproteases.

3. However, this concept cannot be regarded as the "single general inventive concept" required by Rule 13 PCT because it is neither novel nor inventive, since matrix metalloproteases as well as nucleic acids encoding them are known in the prior art (see e.g. WO96/31233; WO03/001983). Methods for increasing sensitivity of stem cells to a chemoattractant are likewise known in the literature (Petit I. et al., nature immunology (2002), vol. 3, pp. 687).
In view of the prior art the first problem to be solved by the present application can be seen in the provision of further methods for increasing sensitivity of stem cells to a chemoattractant. The solution to this problem is the subject of invention 1.
In view of the prior art the second problem to be solved by the present application can be seen in the provision of further nucleic acid constructs encoding a matrix metalloprotease. The solution to this problem is the subject of invention 2.
Due the fact that the common concept cannot be regarded as special technical feature in the sense of Rule 13 PCT and due to the fact that no other "special" technical feature (Rule 13.2 PCT) could be identified to provide a linking concept between the different groups of inventions, each group has to be seen as individual contribution to the art, which is not linked with the other groups by a single general inventive concept. Consequently there is lack of unity.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Invention 1

1. Cited documents

Reference is made to the documents cited in the international search report. The numbering corresponds to the listing of the documents in the international search report:

D1: PETIT ISABELLE ET AL., NATURE IMMUNOLOGY, vol. 3, no. 7, July 2002 (2002-07), pages 687-694
D2: COTTLER-FOX MICHELE H ET AL., HEMATOLOGY / THE EDUCATION

PROGRAM OF THE AMERICAN SOCIETY OF HEMATOLOGY. AMERICAN SOCIETY OF HEMATOLOGY. EDUCATION PROGRAM. 2003, January 2003 (2003-01), pages 419-437

D3: WO 96/31233 A

D4: HEISSIG BEATE ET AL., CELL, vol. 109, no. 5, 31 May 2002 (2002-05-31), pages 625-637

D5: WO 03/001983 A 9 January 2003 (2003-01-09)

D6: JANOWSKA-WIECZOREK ANNA ET AL., EXPERIMENTAL HEMATOLOGY (CHARLOTTESVILLE), vol. 28, no. 11, November 2000 (2000-11), pages 1274-1285

D7: PELED A ET AL., SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, US, vol. 283, 5 February 1999 (1999-02-05), pages 845-848

D8: LAPIDOT TSVEE ET AL., EXPERIMENTAL HEMATOLOGY (CHARLOTTESVILLE), vol. 30, no. 9, September 2002 (2002-09), pages 973-981

2. Subject-matter of the application

Present application relates to stem cells which exhibit increased sensitivity to a chemoattractant. It has been found that hepatic injury (CCl_4 injection) up regulates matrix metalloprotease activity (MMP-2 and MMP-9, = gelatinase A and B) in the liver. An increased level of CXCR4 expression on human MNC cells was also observed in the circulation of CCl_4 treated mice. Moreover supernatants from HT1080, a human cell line which secretes MMP-2 and MMP-9, were found to increase surface CXCR4 expression on enriched human $CD34^+$ cells.

3. Novelty

3.1. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-18, 54, 59-62 is not new in the sense of Article 33(2) PCT.

3.2. D1 discloses (page 690, paragraph bridging left and right hand column) that G-CSF injection up regulates CXCR4 expression on CD34+ cells in vivo. D1 discloses also (page 693, right hand column, lines 8-14) that an increased production of human MMP-2 and MMP-9 by G-CSF-mobilized CD34+ suggests

that these enzymes are involved in the transendothelial migration of immature cells into the periphery and that treatment of mice with a MMP inhibitor prevented G-CSF, SDF- and VEGF mediated mobilization of murine stem cells. D1 discloses moreover (page 691, right hand column, first paragraph) that SDF-1 is cleaved by MMP-2 and MMP-9 to generate a non-functional chemokine, that G-CSF indirectly induced upregulation of CXCR4 expression on BM cells and that this upregulation could be a consequence of the potent collapse of SDF-1 concentrations within the BM. According to the authors up-regulation of CXCR4 may serve to increase the sensitivity of cells to lower SDF-1 signals (page 692, left hand column, first full paragraph, 2nd paragraph). Finally D1 discloses (page 692, right hand column, first paragraph) that SDF-1 increases MMP-9 expression, which causes membrane-bound SCF shedding and release, and that SCF also increases CXCR4 expression and the motility of human CD34+ cells.
Summarising, D1 is considered to disclose that MMP9 increases CXCR4 expression via SCF and that both MMP2 and MMP9 increase CXCR4 expression via SDF-1. D1, thus, anticipates the subject-matter of claims 1-9 and 54.

3.3. D2 discloses (page 423, left hand column, second full paragraph- page 424, left hand column, last paragraph) that stress-released mobilization of progenitors from the BM involves increased production of SDF-1, and proliferation and activation of neutrophils and osteoclasts. Release of proteolytic enzymes, MMP-2 and MMP-9, is followed by shedding of membrane-bound SCF, proliferation of haematopoietic progenitors, increasing surface CXCR4 expression and inactivation of SDF-1, G-CSF, the BM adhesion machinery, and extracellular matrix. D2 thus also anticipates the subject-matter of claims 1-9 and 54.

3.4. D3 discloses the use of gelatinase B (=MMP9) to mobilize haematopoietic stem cells from the bone marrow to the blood for effecting haematopoietic or bone marrow reconstitution. Also disclosed is a pharmaceutical composition comprising the thus mobilized haematopoietic stem cells and its use for effecting haematopoietic or bone marrow reconstitution. The use of MMP9 in the preparation of a pharmaceutical composition for mobilizing haematopoietic stem cells is likewise disclosed. D3 is thus considered to anticipate the subject-matter of claims 10-17, 59, 60, 62.

3.5. D4 discloses that MMP9 releases the stem cell-active cytokine soluble Kit-ligand (sKitL), thereby directing stem and progenitor cell recruitment and facilitating

haematopoietic reconstitution. The authors of D4 found that SDF-1 and VEGF stimulate the release of pro-MMP-9 and induce migration of human CD34+ progenitor and stem cells in a transwell migration assay. The migration of CD34+ cells was completely blocked by addition of MPIs (MMP inhibitors). D4 discloses also culture of stromal cells in serum-free medium containing recombinant active MMP-9 and is thus considered to anticipate the subject-matter of claim 18.

3.6. D5 discloses (claim 22) a pharmaceutical composition comprising an MMP-2 protein and thus anticipates the subject-matter of claims 59-61.

3.7. The subject-matter of claims 19-40 and 55-58 appears to be novel in view of the available prior art.

4. Inventive step

4.1. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 19-40 and 55-58 does not involve an inventive step in the sense of Article 33(3) PCT.

4.2. D6 discloses (paragraph bridging pages 1282 and 1283) that TNF-α enhances chemotaxis of CD34+ cells towards an SDF-1 gradient and up regulates the level of CXCR4 expression in these cells. D6 moreover discloses (page 1282, left hand column, first and second paragraph) that natural and synthetic inhibitors of matrix metalloproteinases were able to block trans-Matrigel migration of CD34+ cells toward SDF-1, indicating that SDF-1 induced trans-Matrigel migration of CD34+ cells is regulated, at least in part, by MMPs. D6 discloses also that SDF-1 stimulated the secretion of MMP-2 and MMP-9 proteins. The subject-matter of claims 22-29, 58 is considered to be obvious in view of the teaching of D6 in particular since D6 also discloses (page 1274, right hand column, first paragraph) that the mirror image of homing is mobilization of HPC from BM to PB and that both these processes require penetration of the subendothelial basal lamina by HPC, which, so the authors of D6 postulate, necessitates the production of matrix-degrading enzymes, especially MMPs.

The subject-matter of claims 19-21 is considered obvious in view of D4.
The subject-matter of claims 30-40, 55-58 is considered obvious in view of the teaching of D2.

Invention 2

1. Cited documents

Reference is made to the documents cited in the international search report. The numbering corresponds to the listing of the documents in the international search report:

D1: PETIT ISABELLE ET AL., NATURE IMMUNOLOGY, vol. 3, no. 7, July 2002 (2002-07), pages 687-694, XP002289815 ISSN: 1529-2908

D2: COTTLER-FOX MICHELE H ET AL., HEMATOLOGY / THE EDUCATION PROGRAM OF THE AMERICAN SOCIETY OF HEMATOLOGY. AMERICAN SOCIETY OF HEMATOLOGY. EDUCATION PROGRAM. 2003, January 2003 (2003-01), pages 419-437, XP002289816 ISSN: 1520-4391

D3: WO 96/31233 A

D4: HEISSIG BEATE ET AL., CELL, vol. 109, no. 5, 31 May 2002 (2002-05-31), pages 625-637, XP002289817 ISSN: 0092-8674

D5: WO 03/001983 A 9 January 2003 (2003-01-09)

D10: HUHTALA P ET AL., JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 266, no. 25, 1991, pages 16485-16490

D10': GenBank accession number

D11: HUHTALA P ET AL., JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 265, no. 19, 5 July 1990 (1990-07-05), pages 11077-11082

D11': GenBank accession number

D12: HEWITT R E ET AL., TRENDS IN GLYCOSCIENCE AND GLYCOTECHNOLOGY, FUJISHIRO, JP, vol. 8, no. 39, January 1996 (1996-01), pages 23-36

D13: WO 99/30730 A1

D14: NAGASE HIDEAKI ET AL., JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 274, no. 31, 30 July 1999 (1999-07-30), pages 21491-21494

D15: SUN H B ET AL., BONE (NEW YORK), vol. 28, no. 3, March 2001 (2001-03), pages 303-309

D16: JANOWSKA-WIECZOREK ANNA ET AL., BLOOD, vol. 93, no. 10, 15 May 1999 (1999-05-15), pages 3379-3390

D17: LOTTI FRANCESCO ET AL., JOURNAL OF VIROLOGY, vol. 76, no. 8, April 2002 (2002-04), pages 3996-4007

D18: GROTE KARSTEN ET AL., CIRCULATION RESEARCH. 13 JUN 2003, vol. 92, no. 11, 13 June 2003 (2003-06-13), pages e80-e86

D19: MAGID RICHARD ET AL., JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 278, no. 35, 29 August 2003 (2003-08-29), pages 32994-32999

2. Subject-matter of the application

Present application relates to a nucleic acid construct comprising a first polynucleotide sequence encoding a matrix metalloproteinase such as MMP-2, MMP-3, MMP-9, MMP-10, MMP-13 and MMP-14, and an inducible cis-acting regulatory element for directing expression in of said polynucleotide in cells. Stem cells, in particular $CD34^{+}/CD38^{-/low}$ haematopoietic stem cells, transformed to express an exogenous polynucleotide encoding a matrix metalloproteinase are likewise disclosed.

3. Novelty

3.1. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 41-46 is not new in the sense of Article 33(2) PCT.

3.2. Claim 41 relates to a nucleic acid construct comprising a first polynucleotide sequence encoding a matrix metalloproteinase or an active portion thereof and an inducible cis-acting regulatory element for directing expression of said polynucleotide in cells.

3.3. D10' discloses the complete structure of the human gene for MMP-9 including the promoter (see Fig. 4 of D10). This sequence also comprises the sequence encoding the signal peptide of MMP-9. Since the MMP-9 promoter comprises a shear stress activation element (see D19, cited as technical evidence only), D10' anticipates the subject-matter of claims 41-46.

3.4. D11' discloses the structure of the human gene for MMP-2 including the promoter (see Fig. 3 of D11). This sequence also comprises the sequence encoding the signal peptide of MMP-2. Since the MMP-2 promoter comprises a shear stress activation element (see D18, cited as technical evidence only), D11' anticipates

the subject-matter of claims 41-46.

3.5. The subject-matter of claims 47-53 appears to be novel in view of the available prior art.

4. Inventive step

4.1. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 47-53 does not involve an inventive step in the sense of Article 33(3) PCT.

4.2. Claim 47 relates to a cell-line comprising stem cells transformed to express an exogenous polynucleotide encoding a matrix metalloproteinase.

4.3. D1 discloses that MMP-2 and MMP-9 are involved in the transendothelial migration of immature CD34+ cells from the bone marrow into the periphery (page 693, left hand column, first paragraph). D2 also discloses that MMP-9 is involved in stem cell mobilization (page 421, right hand column, first paragraph). D3 discloses the use of gelatinase B (=MMP9) to mobilize haematopoietic stem cells from the bone marrow to the blood for effecting haematopoietic or bone marrow reconstitution (page 10, line 15 - page 11, line 14). D4 discloses that induction of MMP-9 in bone marrow cells leads to release of KitL, permitting the transfer of endothelial and haematopoietic stem cells form the quiescent to proliferative niche. D5 discloses the recombinant expression of MMP-2 in a variety of tissues including haematopoietic tissue by using haematopoietic stem cell differentiation factor promoters (page 19, first full paragraph). D16 discloses that peripheral blood $CD34^+$ cells, regardless of whether they are mobilized or not, strongly express both MMP-2 and MMP-9 in contrast to steady state bone marrow $CD34^+$ cells, which did not. Positive correlations were established between expression of MMP-2 and MMP-9 and $CD34^+$ cell migration (abstract).

In view of this prior art the provision of cell-line comprising stem cells transformed to express an exogenous polynucleotide encoding a matrix metalloproteinase becomes obvious to the skilled person.

4.4. Dependent claims 48-53 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in

respect of inventive step in view of the disclosure of any of D1, D2, D3, D4, D5 or D16.